B12 tampons, non-woven fabrics, microspheres, nanospheres, gauzes, gels or guide channels.

B13 19. (amended) The industrial or medical articles or devices coated with the haemocompatible material according to any of claims 9, 10, 11, 12, 13, 14, 15 or 16 wherein said articles or devices are selected from the group consisting of catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone replacements or cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for cell and tissue regeneration, supports for peptides, proteins and antibodies.

REMARKS

In response to the objection set forth in paragraph 1 on page 2 of the Office Action, an Abstract, which has been prepared in conformance with 37 CFR §1.72(b), is being submitted on a separate sheet of paper.

In paragraph 2 of the Office Action, the Examiner objected to the reference to a foreign application as the basis for the incorporation by reference of essential material. In response, the applicant has amended the specification to insert U.S. patent 6,051,701. A declaration attesting to this fact is attached to this Amendment. The specification has also been amended to include the subject matter that has been incorporated by reference from WO98/45335. A declaration attesting to the identity of the subject matter that has been added to the specification with the subject matter of the reference published application is attached to this Amendment. For these reasons, it is requested that this ground of rejection be withdrawn.

In paragraph 4 and 5 of the office Action, claims 1-19

were rejected under 35 U.S.C.§ 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant that the inventor at the time the application was filed had possession of the claimed invention.

Reconsideration is requested in view of this Amendment.

The amended specification now includes a specific reference to a prior United States patent and recites detailed information which has been based on WO98/45335. This information enable one who is skilled in the relevant art to make and use the claimed invention.

The Examiners objection to claim 19 in paragraph 5 of the office Action is noted. Claim 19 has been amended to point out that the invention is used to coat such devices, not to produce such devices stated in the claim. In addition claim 19 has been revised to be a multiple dependent claim which is dependent on claims 9-16.

For the above stated reasons, it is requested that the rejection to claims 1-19 under 35 U.S.C § 112, first paragraph, be withdrawn.

In paragraph 6 of the Office Action, claims 2-8, 12-17 and 19 are rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention.

Reconsideration is requested.

In response claims 2, 3, 4 and 5 have been rewritten in independent form to point out the invention. A proper antecedent basis has been added for the terms "polyurethane" and "sulphated hyaluronic acid". The spelling of the term "aliphatic" is noted and has been corrected.

The term "series" has been deleted form claim 5 and the text of the claim has been revised to clearly point out that the carboxylic function is being esterified with an alcoholic function.

The Examiner is asked to reconsider the objection to the uses of the term "spacer chain". This term is used in the art

to define well known chemical groups by the manner that function as a bridge in a chemical molecule. The groups that are defined by this term are finite and can readily be identified by those who are skilled in the art.

Claims 7 and 8 have been rewritten to include a proper antecedent basis for each of the terms. The structural formula for each of formulas I, IV, III etc. have been inserted. In response to the Examiner's objection to the use of the term "their fragments", the applicants have deleted this term. In addition the terms "semisynthetic" and "copolymers of the same or their derivatives" have been deleted from the claims. Claims 14, 15, 16 and 19 have been amended to clearly point out the invention. Each occurrence of the term "such as" has been deleted. Claim 17 has been amended to reference the species in the alternative. Claim 19 has been amended to point out that the recited articles and devices are "coated" with the materials defined in the referenced claims. This amendment to Claim 19 is supported by the specification at page 6, line 13 of the specification.

The terms "soft tissue prostheses" and "prostheses of animal origin" as used in Claim 19 were noted by the Examiner as being unclear. The term "soft tissue"reefers in general to non-bone tissue such as nerves, veins, organs and glands. The terms "soft tissue prostheses" includes a variety of prostheses such as synthetic graft materials for use in the repair of blood vessels and other organs. The term "prostheses of animal origin" includes body parts from animals that are used in humans such as valves from pigs that are used for replacement of cardiac valves. For these reasons, it is believed that the amended claims are in proper form and that the rejections under 35 U.S.C.§112, second paragraph be withdrawn.

In paragraph 8 of the Office Action, Claims 1-19 were rejected under 35 U.S.C. 103(a) as being unpatentable over Balazs et al. '865 (Balazs) in view of WO 95/25751 (Cialdi et al.) and Halpern et al. '114 (Halpern).

Reconsideration is requested.

The present invention is concerned with polymers that are highly stable and possess good mechanical characteristics such as resistance to wear, bending, elastic deformation and also possess anticoagulant and non-thrombogenic properties which inhibit platelet adhesion and aggregation. These properties are necessary if a particular material is to be used for making biomaterials or for coating biomaterials.

The present applicants have discovered that the claimed polymers which are polyurethanes which are covalently linked to sulphated hyaluronic acid or derivatives of sulphated hyaluronic acid possess both stable anti-coagulant properties as well as mechanical properties that make the polymers useful as biomaterials.

Balazs teaches us polymeric materials modified with hyaluronic acid or a salt thereof but does not mention sulphated hyaluronic acid derivatives. In addition, Balazs describes introducing the hyaluronic acid by mixing or by coating. There is no mention of covalently bound hyaluronic acid in the Balazs patent. Cialdi discloses sulphated hyaluronic acid materials but does not mention the coating of polyurethanes or the covalent binding of any material.

However, nothing in the cited prior art suggests in any way, that the polyurethane should be covalently bound to the hyaluronic acid derivatives as described and claimed in the present application. As noted above, the polymeric materials disclosed by Balazs are modified with hyaluronic acid or a salt thereof, but not with sulphated derivatives.

It could not be predicted from the teachings of Cialdi that the disclosed sulphated derivatives would retain their anti-coagulant properties if they were covalently bound to a polyurethane polymer so that a person skilled in the art would a priori be apprised of the anti-coagulant properties of the claimed polymers of the present invention.

The Examiner is asked to reconsider the application of the Halpern reference. This patent deals with polysaccharides which are covalently bound to a substrate. It does not mention polyurethanes or sulphated hyaluronic acid materials. For

these reasons, it is requested that this ground of rejection be withdrawn.

Authorization is hereby given to charge any additional fee to Deposit Account No. 08-1540.

An early and favorable action is earnestly solicited.

Respectfully Submitted

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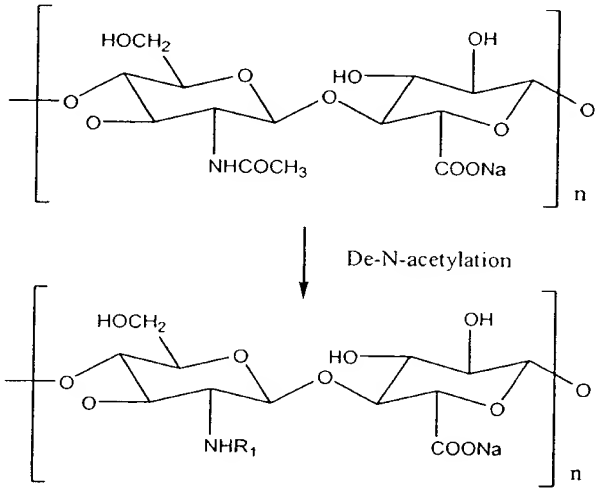
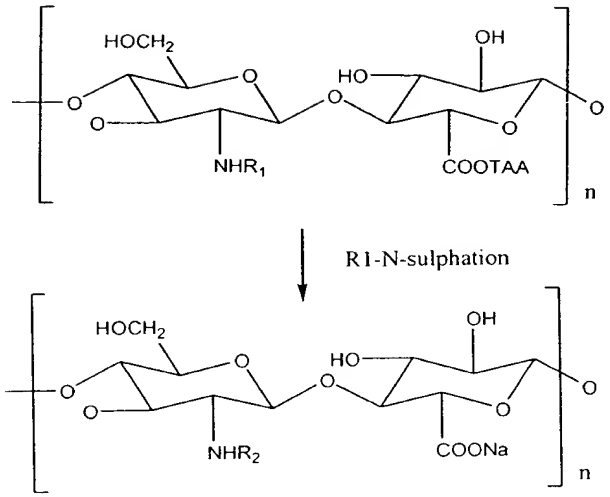
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on 9/4/01

Marked up copy of amendments to specification:

Page 3, line 21, delete the paragraph at lines 21-29 and insert the following:

By sulphated hyaluronic acid and sulphated hyaluronic acid derivatives, we mean:
A_1) O-sulphated hyaluronic acid, and
B_1) O-sulphated hyaluronic acid derivatives,
both types being disclosed in [WO 95/25751] U.S. Patent No. 6,051,701, [we] which is incorporated herewith by reference;
B_1) N-sulphated hyaluronic acids, and
B_2) N-sulphated hyaluronic acid derivatives,
both types being [disclosed in WO 98/45335, we incorporate by reference] obtainable by means of a controlled sulphation reaction on the amino group of glucosamine of hyaluronic acid, previously deacetylated according to the procedure described by P. Shaklee (1984) Biochem. J., 217, 187-197. The reaction proceeds as illustrated below:





n: from 12 to 12,500

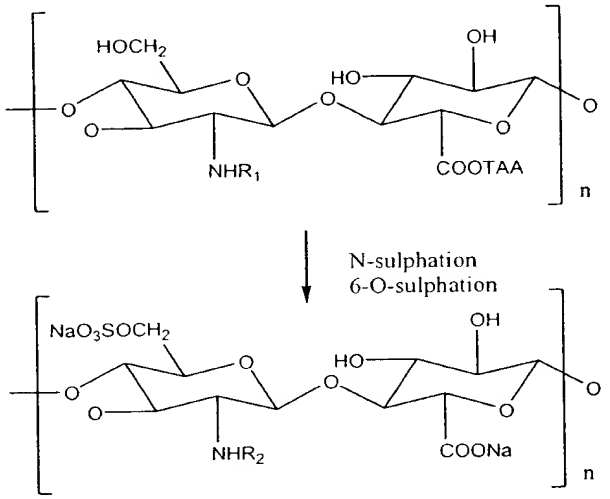
R_1 = H, $COCH_3$

TAA = tetra-alkylammonium

R_2 = SO_3, $COCH_3$

Diagram 1

b) and c) mean the products of the chemical reaction illustrated in Diagram 1, wherein, besides the amino group of glucosamine, the primary hydroxy function of the same residue is also totally or partially involved in the sulphation reaction, as illustrated below:



n: from 12 to 12,500

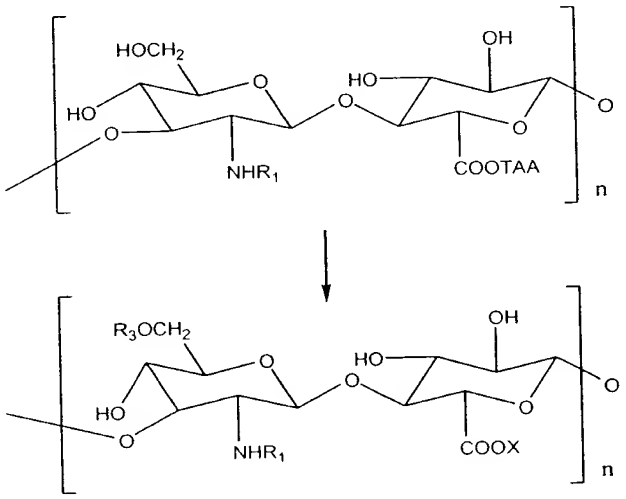
R_1 = H, $COCH_3$

TAA = tetra-alkylammonium

R_2 = SO_3, $COCH_3$

Diagram 2

The derivatives generated according to diagrams 1 and 2 can be used as intermediate reactants in the preparation of compounds, according to the procedure described in U.S. 4,851,521, wherein the carboxy function of the glucuronic residue of hyaluronic acid, partially 2-N-sulphated or partially 2-N-sulphated and partially or totally 6-O-sulphated, is partially or completely reacted with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, producing the respective partial or total esters:



n: from 12 to 12,500

R_1 = H, $COCH_3$

TAA = tetra-alkylammonium

R_2 = SO_3, $COCH_3$

R_3 = SO_3, H

X = alcoholic residue, Sodium

Diagram 3

Moreover it is possible to use the synthetic derivatives according to diagrams 1 and 2 as intermediates in the preparation of crosslinked compounds, according to the procedures described in U.S. 5,676,964 and U.S. 4,957,744 respectively, wherein a part or all of the carboxy groups belonging to the D-glucosamine residue are reacted: i) using condensing agents with the alcoholic functions of the same polysaccharide chain or other chains, generating inner (or lactone) esters and intermolecular esters; ii) with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating crosslinking by means of spacer chains.

The above said sulphated compounds obtained according to the process of the present invention can be optionally salified with heavy metals, the heavy, metals being selected from the group of metal elements in th 4^{th}, 5_{th} and 6^{th} periods of the periodic table, such as silver, iron, cobalt, copper, zinc, arsenic, strontium, zirconium, antimony, gold, cesium, tungsten, selenium, platinum, ruthenium, bismuth, tin, titanium and mercury.

Page 4, after line 26, insert:

The process for the preparation of the compounds B_1 and B_2 mainly consists of two steps, the first involving the controlled deacetylation of the natural polysaccharide, and the second involving the specific sulphation reaction of the primary hydroxyl or free amino functions of glucosamine. Fractions of hyaluronic acid from biological and fermentation sources, with a molecular weight of between 5,000 and

5,000,000 Da, preferably between 50,000 Da and 300,000 Da, are solubilized in hydrazine hydroxide with a purity of no less than 98%, in a concentration range of between 1 and 50mg/ml, preferably between 5 and 25 mg/ml. This solution is then supplemented with hydrazine sulphate in a weight/volume concentration varying between 0.1 and 3%, preferably 1%. The reaction is conducted within a temperature range of 40 to 90°C, preferably 60°C, under agitation, for as long as it takes to reach the desired degree of N-deacetylation.
Table 1 hereafter reports the yield expressed as the percentage of free amino groups, in terms of time expressed as hours of reaction:

Table 1

Test	Temperature (°C)	Time (hours)	N-deacetylation (%)*
DAc 1**	60°C	4	3
DAc 2	60°C	8	5
DAc 3	60°C	16	9
DAc 4	60°C	24	14
DAc 5	60°C	48	23
DAc 6	60°C	72	36

* The percentage of N-deacetylation is determined according to the method of J. Riesenfeld (Analy. Bioch. 1990, vol. 188, pages 383-389).
** DAc + N-deacetylation

The reaction is then stopped by precipitation with a polar solvent, preferably ethanol. The precipitate is partially vacuum-dried and treated with a solution of iodic acid with a molarity range of between 0.1 and 1M, preferably 0.5M, and lastly, with iodohydric acid at a concentration of 57% (w/v). The pH of the solution is maintained between 5 and 7 by adding a solution of sodium acetate (10% w/v).

The aqueous phase containing the modified polysaccharide is extracted by repeated treatments with diethylether and then,

once the yellow color has completely disappeared, the solution is treated again with ethanol.

The precipitate which forms, after further drying at 40°C, is solubilized in water at a concentration of between 10ng/ml and 40 ng/ml, preferably 25 ng/ml, and the solution is percolated through a column containing an ion exchange resin activated with a tetra-alkylammonium hydroxide, where the alkyl residue of the quaternary ammonium is constituted by a chain of between 1 and 4 carbon atoms; tetrabutyl-ammonium hydroxide is preferably used.

The percolated product, represented by the quaternary ammonium salt of the modified polysaccharide, is then freeze-dried.

Preparation of: a) partially N-sulphated derivative (Method A)

The quaternary ammonium salt, preferably of tetrabutyl-ammonium, of the partially deacetylated polysaccharide, is solubilized in a polar a solvent such as dimethyl sulphoxide, dimethyl formamide, dimethyl acetamide, N-methyl-pyrrolidone, preferably dimethyl formamide (DMFA), at a concentration of between 5 and 50mg/ml (preferably 25mg/ml).

The organic solution is supplemented with another solution obtained by a sulphating complex constituted by dimethylformamide sulphotrioxide (DMFA-SO_3), in DMFA, at a concentration varying between 50 and 200 mg/ml and preferably 100mg/ml. The quantity of complex to be used, expressed in moles of SO_3, proves surprisingly to be equivalent to the moles of amino groups released by the N-deacetylation reaction.

The sulphation reaction proceeds at a temperature of between 0° and 20°C, preferably 4°C, for no longer than 4 hours and is then stopped by adding cold, distilled water.

The reaction solvent is first purified by precipitating the

partially N-sulphated hyaluronic acid with ethanol and then dialyzing the resolubilized product with distilled water.

Lastly, the solution is freeze-dried and the solid product thus obtained undergoes chemical-analytical characterization to determine the degree of N-sulphation and the mean molecular weight (Table 2).

Table 2

Test	% deacetylation	% N-sulphation	mean MW (Da)
HA	0	0	165,000
HA-NS1	5.0(DAc2)	4.8	157,000
HA-NS2	14.2(DAc4)	13.9	147,000
HA-NS3	23.5(DAC5)	23.0	139,000
HA-NS4	36.1(DAc6)	34.2	124,000

HA = hyaluronic acid
HA-N-S = N-sulphated hyaluronic acid

Preparation of: b) partially 2-N-sulphated derivative (Method B)

The quaternary ammonium salt, preferably of tetrabutyl-ammonium, of the partially N-deacetylated polysaccharide is solubilized in a polar solvent such as dimethylsulphoxide, dimethylformamide, dimethylacetamide, N-methyl-pyrrolidone, preferably dimethylformamide (DMFA), at a concentration of between 54 and 50mg/ml, preferably 30 mg/ml.

The organic solution is supplemented with another solution obtained by solubilizing the sulphating complex constituted by dimethylformamide sulphotrioxide (DMFA-SO_3), in DMFA, at concentrations varying between 50 and 200 mg/ml and preferably 100 mg/ml. The quantity of complex used, expressed as moles of SO_3 prove surprisingly to be equivalent to the moles of amino groups released by the N-deacetylation reaction.

The sulphation reaction proceeds at a temperature of between 0° and 20°C, preferably at 4°C for 4 hours. A solution prepared by solubilizing the pyridine-sulfotrioxide complex in dimethylsulphoxide in such a quantity that the ratio between the moles of SO_3 of the sulphating agent and the moles of $-CH_2OH$ is between 1.1 and 1.3. Larger quantities of reagent may favor any substitution reactions in other alcohol groups (secondary) of the polysaccharide chain.

The reaction the proceeds for another 16 hours at least after which it is stopped by adding cold distilled water.

All subsequent steps concerning the purification of the modified polysaccharide are those described in Method A.

The analytical characterization performed on the derivatives obtained confirmed that the sulphation method proves surprisingly not only to substitute all the amino groups obtained by the partial deacetylation, but also results in the complete substitution of the primary alcohol group of the glucosamine residue of hyaluronic acid (Table 3).

Table 3

Test	% N-deacetylation	% N-sulphation	% 6-O-sulphation
HA-N-OS1	5.0(DAc 2)	4.8	100
HA-N-S1	14.2 (DAc 4)	13.9	99.2
HA-N-O-S1	23.5 (DAc 5	23.0	98.9
HA-N-O-S1	36.1 (DAc 6)	34.2	96.5

Moreover, by varying the molar quantities of the pyridine-SO_3 complex according to the primary hydroxyl groups (molar ratio of between 0.1 and 1), Method B enables a series of partially 2-N-sulphated and partially 6-O-sulphated derivatives to be obtained.

Marked up copy of the amended claims:

2. (amended) The polyurethane according to claim 1, wherein the [starting] said polyurethane comprises the repeating unit 4,4'-methylenebis (phenylisocyanate).

3. (twice amended) The polyurethane according to claim 1, wherein the [starting] said sulphated hyaluronic acid is selected from the group consisting of:

A_1) O-sulphated hyaluronic acid, and

B_2) N-sulphated hyaluronic acid.

4. (twice amended) The polyurethane according to claim 1, wherein the [starting] said sulfated hyaluronic acid derivative is selected from the group consisting of:

A_1) O-sulphated hyaluronic acid, and

B_2) N-sulphated hyaluronic acid.

5. (amended) The polyurethane according to claim 4, wherein the hyaluronic acid derivatives used to prepare the [starting] said sulphated hyaluronic acid A_2 and B_2 are selected from the group consisting of:

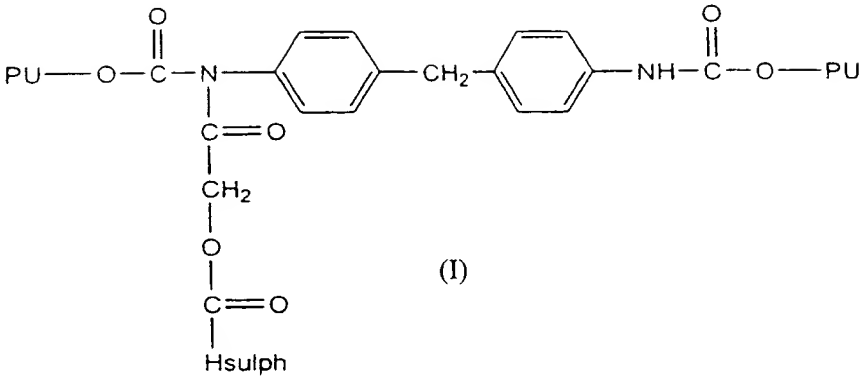
the partial esters of hyaluronic acid containing at least one free carboxylic function and the remaining carboxylic function esterified with [alcohols of the] an aliphatic [fatic], aromatic, arylaliphatic, cycloaliphatic or heterocyclic alcohol [series], and

the partial crosslinked esters containing at least one free carboxylic function and the remaining carboxylic functions are esterified with the alcoholic function of the same hyaluronic acid molecule or of a different [chain] hyaluronic acid molecule,

the partial crosslinked esters containing at least one free carboxylic function reacted with an [polyalcohol of the] aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic [series] polyalcohol, and wherein cross linking is thereafter generated by means of spacer chains.

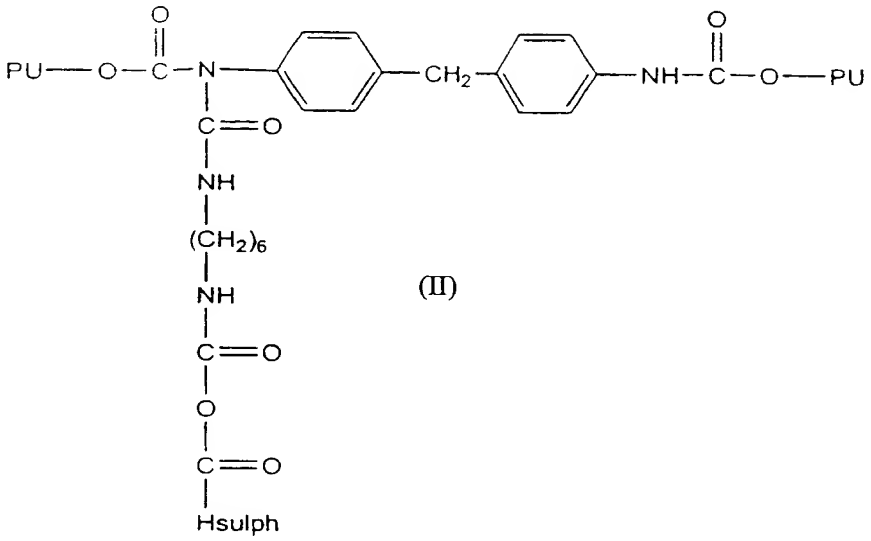
6. (twice amended) The polyurethane according to claim 1

of formula(I)



(I)

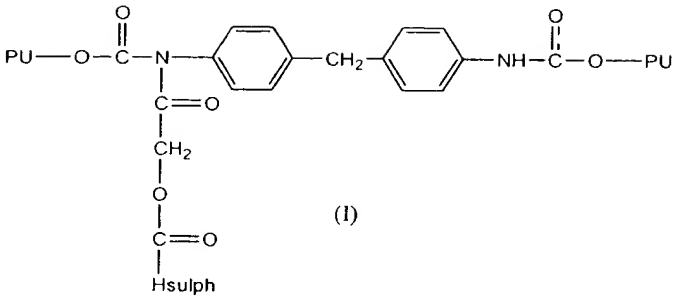
or formula (II)



(II)

wherein PU is a residue of the polyurethane chain, Hsulph is a residue of the sulphated hyaluronic acid or a residue of a sulphated hyaluronic acid derivative containing a[t] [least one] free carboxylic function.

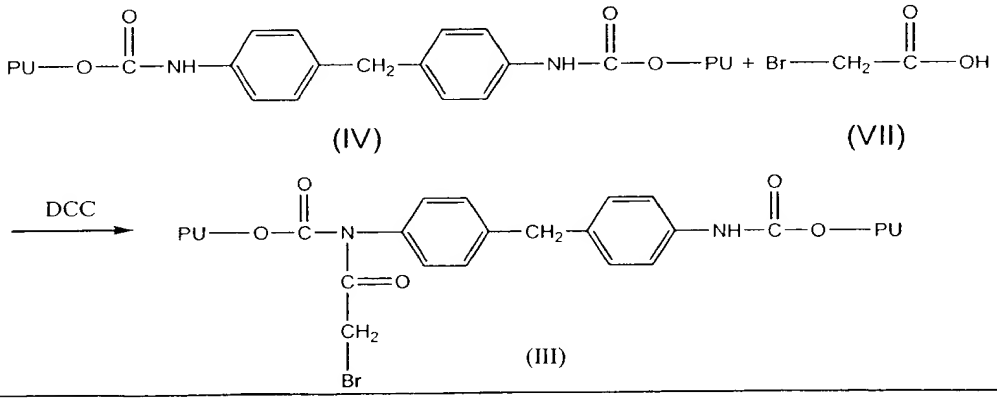
7. (amended) A process for preparing the polyurethane of formula(I)



(I)

wherein PU and Hsulph are as defined in claim 6,
comprising the following steps:

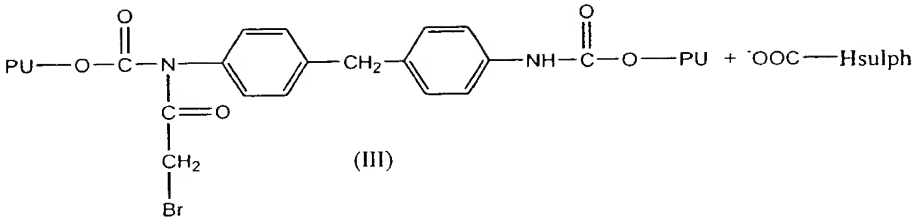
i) the polyurethane (IV) is reacted with bromoacetic acid (VII) in the presence of N,N'-dicyclohexylcarbodiimide (DCC), to obtain the adduct of formula (III) [;]



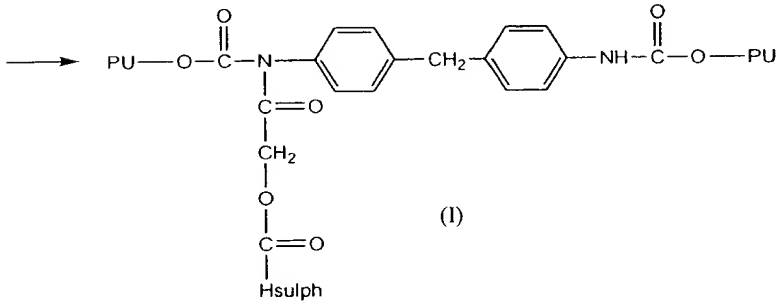
(IV) (VII)

(III)

ii) the adduct (III) coming from step i) is reacted with HOOC-Hsulph, wherein Hsulph is defined as above, thereby obtaining the compound of formula (I)

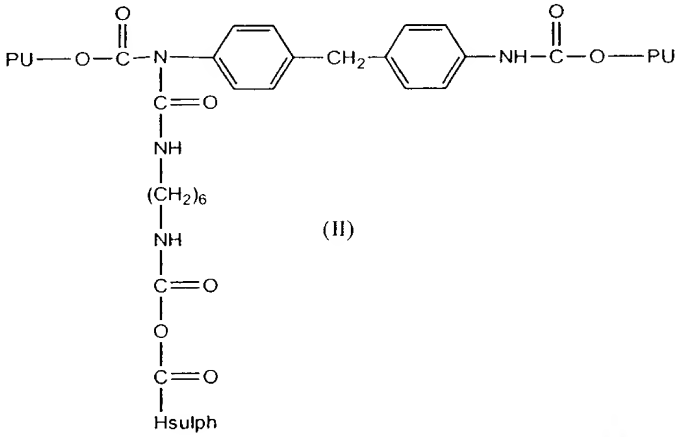


(III)



(I)

8. (amended) A process for preparing the polyurethane of formula (II)

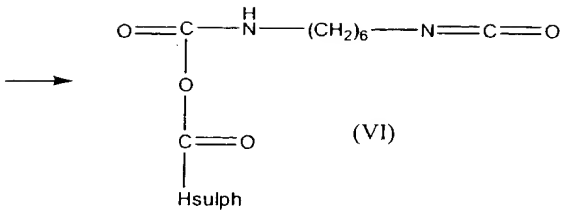


(II)

wherein PU and Hsulph are as defined in claim 6, comprising the following steps:

i') HOOC-Hsulph is reacted with hexamethylenediisocyanate (HMDI) (V), to obtain the adduct [of] formula (VI)

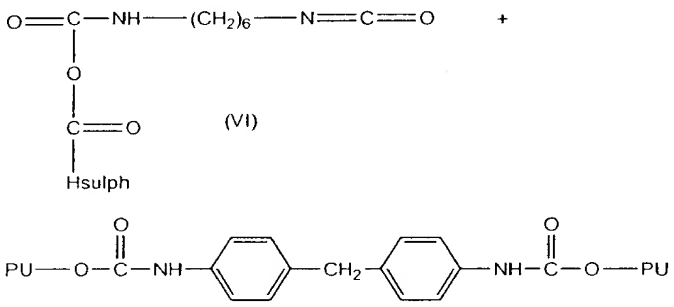
$O=C=N-(CH_2)_6-N=C=O$ + sulphH-COOH (V)



(VI)

wherein Hsulph is defined as above;

(ii') the adduct (VI) coming from step i') is reacted with the polyurethane (IV) to obtain the [desired product] said polyurethane of formula (II)



(VI)

(IV)

12. (amended) The haemocompatible material according to claim 11, wherein said pharmaceutically active substance is selected from the group consisting of antibiotics, anti-infective, antimicrobial, antiviral, cytostatic, antitumoral, anti inflammatory, wound healing agents, anesthetics, cholinergic or adrenergic agonists or antagonists, antithrombotic, anticoagulant, haemostatic, fibrinolytic, thrombolytic agents, proteins [or their fragments], peptides, polynucleotide, growth factors, enzymes and vaccines.

13. (twice amended) The haemocompatible material according to claim 9 further comprising at least one natural, synthetic [or semisynthetic] polymer.

14. (amended) The haemocompatible material according to claim 13, wherein said natural polymer is selected from the group consisting of collagen, collagen coprecipitates and glycosamino glycans, cellulose, polysaccharides in the form of [gels such as] chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or the alginates, polymannan or polyglycans, starch and natural gums.

15. (amended) The haemocompatible material according to claim 13, wherein said [semisynthetic] polymer is selected from the group consisting of collagen cross linked with [agents such as] aldehydes [or precursors of the same], dicarboxylic acids or their halides, diamines, derivatives of cellulose, hyaluronic acid, chitin or chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum and glycosamino glycans.

16. (amended) The haemocompatible material according to claim 13, wherein said synthetic polymer is selected from the group consisting of polylactic acid, polyglycolic acid [or copolymers of the same or their derivatives], polydioxanes, polyphosphazenes, polysulphonic resins and PTFE.

17. (twice amended) The haemocompatible material according to claim 9, in the form of sponges, films, membranes, threads, tampons, non-woven fabrics, microspheres, nanospheres, gauzes, gels [and] or guide channels.

19. (amended) The industrial or medical articles or devices coated with the haemocompatible material according to any of claims[18] 9, 10, 11, 12, 13, 14, 15 or 16, wherein said articles or devices are selected from the group consisting of catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone replacements or cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for cell and tissue regeneration, supports for peptides, proteins and antibodies.